MMHCC Lung Cancer Organ Site

Erica L. Jackson and Tyler Jacks

Welcome to the MMHCC Lung Cancer Site. As you enter the site you will find an introductory section providing background information on lung cancer and a general overview of current methods for diagnosis and treatment, molecular alterations occurring in lung cancer, and existing murine models of lung cancer. The introductory section will be followed by several sections providing more in depth discussion of the topics listed below.

- Introduction/Overview
- Human Lung Cancer
  - Lung Development and Biology (Human and Mouse Comparative)
  - Tumor Classification and Staging
  - Molecular Alterations
  - Novel Therapeutics
- Murine Lung Cancer
  - Murine Models of Lung Cancer
  - Classification of Murine Lung Tumors

Introduction

Lung cancer is the leading cause of cancer deaths worldwide, with 169,500 new cases and 157,400 deaths predicted for 2001 in the United States alone (32). The majority of lung cancer cases are related to tobacco use with approximately 10% of lung tumors arising in non-smokers (7). However, the incidence of lung cancer deaths that are not associated with smoking or other environmental factors is increasing at a higher rate than in any other group (54).

Lung cancer patients suffer a high case: fatality ratio with a 5 year survival rate of ~14%. For treatment purposes lung cancer is divided into two histopathologic classes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which differ in their responses to therapy. Approximately 80% of lung cancer cases are classified as NSCLC, while SCLC accounts for ~18% (80). The two classes of lung cancer are characterized by distinct patterns of oncogene activation, tumor suppressor gene mutation and chromosomal alterations, which may explain their different biologies. Little progress has been made in the treatment of lung cancer over the past 30 years. Since 1970 to the present, the 5 year survival rate has only increased from 7 to 14%.

The development of murine models of lung cancer may aid in our understanding of lung tumor biology and facilitate the development and testing of novel therapeutic approaches and methods for early diagnosis. To this end, mouse models should mimic the genetic alterations found in human lung tumors, the histological characteristics of human tumors or both. To date, several approaches have been taken in the development of murine lung cancer including chemically induced tumors, transgenic strains expressing relevant oncogenes, and strains in which important tumor suppressor genes have been knocked out. More recently, mice carrying latent and conditional alleles of oncogenes

and tumor suppressor genes known to be mutated in human lung cancer have been developed. These models more accurately mimic the human situation in which genetic mutation occurs in a subset of cells within adult somatic tissues.

Diagnosis and Treatment

Chest x-ray and sputum cytology are regularly used to detect lung cancer in symptomatic patients. Recent data suggest that low radiation dose spiral CT is capable of detecting early abnormalities in lungs of high risk individuals, but it remains to be seen whether this screening method will result in a reduction in lung cancer mortality (56). The ultimate diagnosis of both SCLC and NSCLC is based on fiber-optic bronchoscopy and histological analysis of sputum or biopsy samples.

SCLCs are neuroendocrine (NE) tumors of highly aggressive nature (Embed histology example here). Often the cancer has metastasized to distant organs by the time of diagnosis. The size of the primary lesion and extent of metastasis dictate the treatment regimen. Due to its propensity for metastasis, SCLC is rarely treated by surgical resection (30). Combination chemotherapy regimens that include a platinum agent are the standard of care for most SCLCs, with the PE regimen (cisplatin and etoposide) being the most commonly used in the US. Although most SCLCs are initial highly responsive to therapy, typically the primary tumor or metastasis become resistant to chemotherapy and >90% of patients succumb to the disease. For more information on treatment options for SCLC please see: Small Cell Lung Cancer (PDQ®): Treatment (www.cancer.gov)

NSCLCs can be further subclassified into squamous cell carcinoma, adenocarcinoma and large cell carcinoma, with adenocarcinoma being the most common (histology for each subclass will be shown). However, for treatment purposes NSCLC is considered a uniform group of aggressive cancers, and again treatment options are based on the stage of disease at the time of diagnosis. Surgery and radiotherapy are used to treat early-stage disease. For patients with unresectable metastatic disease, platinum based combination chemotherapy is again the treatment of choice. Recent randomized trials demonstrate that as long as the therapy contains a platinum compound (cisplatin or carboplatin) and a modern agent active against NSCLC (paclitaxel, docetaxel, gemcitabine or vinorelbine) the survival benefits are the same (16). The addition of concurrent radiotherapy to the PE regimen may also provide some additional survival benefits. However, most patients become resistant to therapy, relapse and die from the disease. For more information regarding treatment options for NSCLC see: Small Cell Lung Cancer (PDQ®): Treatment Option Overview (www.cancer.gov)

For both SCLC and NSCLC patients the performance status of the individual is considered when choosing a course of treatment. Platinum compounds have poor toxicity profiles and may not be suitable for all patients. Due to the high rate of relapse and toxic side affects under the standard therapies many new therapeutic strategies are being developed. Advances in the understanding of the molecular events underlying the development of lung cancer have enabled researchers to develop rationally targeted therapies. These biologic agents specifically target proteins used by cancer cells to promote inappropriate growth and survival. Such agents include selective protein kinase inhibitors, a variety of antisense oligonucleotides, and antibodies all used to inhibit the expression or function of growth factor receptors, signal transduction proteins or anti-

apoptotic mediators. The efficacy of such agents is still being evaluated but targeted therapeutics may provide treatment options with increased efficacy and decreased toxicity. Genetically modified murine lung cancer models may provide a useful reagent for pre-clinical testing of therapeutics directed against the specific molecular lesions driving tumorigenesis in these mice.

Molecular Characterization of Human Lung Cancer Overview

Lung cancer arises as the result of numerous genetic lesions often caused by exposure to cigarette smoke or other environmental carcinogens. It is thought that 10 or more genetic or epigenetic abnormalities must occur before a lung tumor becomes clinically evident (63). Genetic alterations can occur at the chromosomal level including large gains and deletions, at the nucleotide level, or through epigenetic changes such as DNA methylation. The changes that occur result in the activation of oncogenes and other growth promoting genes, and in the inactivation of tumor suppressor genes. NSCLC and SCLC exhibit distinct but overlapping patterns of genetic alterations.

Oncogene/Growth promoters

The protein products of oncogenes are involved in processes that stimulate cellular proliferation or survival. During tumorigenesis oncogene activation can occur through point mutations resulting in constitutively active proteins, through gene amplification and through over expression. In addition, the acquisition of growth promoting autocrine loops, in which individual tumor cells express both growth factors and their cognate receptor. Several oncogenes and growth promoting factors are known to be altered in NSCLC including K-ras, Erb-B1 (EGFR), Erb-B2 (HER-2/neu), myc, raf, bcl-1, bcl-2 and cyclin D1 (Reviewed in(95), (97)). SCLCs are characterized by activation or over expression of myc, raf, myb, Erb-B1, Bcl-2, fms, rlf and by Kit/SCF and GRP/GRP receptor co-expression (Reviewed in (95), (81)).

Tumor Suppressor Genes(TSGs)

The protein products of tumor suppressor genes are involved in the inhibition of cell growth or survival. Several TSGs have been found to be deleted or mutationally inactivated in lung cancer. In addition, several chromosomal regions are specifically deleted in tumors, suggesting the presence of unidentified TSGs in these locations. LOH of p53 is frequent in both SCLC and NSCLC. RB mutations are common in SCLC; they are less frequent in NSCLC. However, mutations of $p16^{INK4}$, a regulator of RB function, are found in many NSCLCs (81). In addition, deletions of several regions of chromosome 3p, 4q, 8p, 9q, Xp, Xq are found in both SCLC and NSCLC. Several regions, including 6q, 9p and 19p are frequently lost in NSCLC, while deletions of 5q, 10q and 22q are unique to SCLC (28). Many additional regions of allelic loss are found at lower frequencies in lung tumors. Candidate TSGs have been proposed for some regions, but in others the underlying TSGs remain to be discovered.

Overview of Existing Mouse Models

Murine lung cancer models provide opportunities to characterize the serial stages of tumor progression and to investigate the molecular alterations associated with them. In addition mouse models allow for the testing of novel chemopreventives, therapeutics and screening methods. The original models include spontaneous and carcinogen induced tumors in sensitive mouse strains such as A/J and SWR. A broad range of chemicals can induce the development of adenomas/adenocarcinomas in susceptible strains including tobacco smoke (106), urethane, metals and individual constituents of tobacco smoke such as polyaromatic hydrocarbons and nitrosamines (Reviewed in Stoner 1998 (86) and Tuveson 1999 (97)). These murine adenocarcinomas contain certain molecular alterations observed human lung carcinomas including K-ras mutations (~90%) and LOH of chromosomal regions containing the murine p16Ink4a gene. Of note, the only existing murine models of squamous cell carcinoma were created either by direct application of carcinogen through intratracheal instillation or by rigorous topical application of carcinogen. Based on an understanding of the molecular alterations that occur in both murine and human lung tumors, several transgenic mouse NSCLC models have been created.

More recently mice carrying latent or conditional alleles of oncogenes and tumor suppressor genes have been created in order to more closely model the human situation in which tumorigenesis is initiated through somatic mutations occurring in adult tissues. Alleles of tumor suppressor genes flanked by loxP sites (floxed alleles) are expressed normally in the germline configuration, but after expression of Cre recombinase in the cell all or a critical portion of the gene is deleted leading to its inactivation. Furthermore, several inducible mouse lung adenocarcinoma models have been created using the doxycycline regulatable tet-operator system, which provides a unique opportunity to study the genes required for tumor maintenance and thus may help to identify potential therapeutic targets.

THE SECTIONS BELOW ARE FROM THE LINKS ABOVE AND PROVIDE MORE IN DEPTH DISCUSSIONS OF THE TOPICS

Lung Development and Biology

There are five stages of human lung development: the embryonic period, occurring between 3 and 6 weeks of gestation; the pseudoglandular period, from 6-16 weeks of gestation; the canalicular period, from 16-28 weeks; the saccular period from 28-36 weeks; and the alveolar period, from 36 weeks of gestation to about 4 years of age (10). Fetal lung development begins with the formation of the primitive lung bud that arises from the foregut, and is first seen as a groove in the floor of the primitive pharynx evaginates into distinct laryngotracheal diverticulum and then elongates caudally into the primitive mesenchyme. The lung bud is lined with endodermally derived epithelium. Formation of the major airways occurs during the embryonic period through a dichotomous branching of the diverticulum(101).

Budding and branching of the airways continues through the pseudoglandular period and results in the formation of the small airways including the terminal bronchioles. Budding and branching occur in response to stimuli produced in the surrounding mesoderm. By 16 weeks of gestation the branching of conducting portion of the

tracheobronchial tree is complete, resulting in a bilobed left lung and a trilobed right lung. At this time four of the eight bronchiolar epithelial cell types comprising the mature lung are recognizable: ciliated, non-ciliated (pre-Clara), secretory and early basal (37).

The respiratory structures and surrounding vasculature of the lung begin to develop during the canalicular phase. Capillaries are positioned in close proximity to the airway epithelium in order to allow future gas exchange. The primitive respiratory bronchioles arise from the terminal bronchioles and contain terminal sacs in their walls, that will later give rise to alveoli.

Development of the alveoli begins in the saccular phase with the subdivision of saccules, terminal clusters of airways. These primitive alveoli are pouches in the walls of saccules and respiratory bronchioles and are lined by type I cells. Development of true vascularized alveoli occurs in the alveolar phase. These alveoli are lined by type I cells and also contain alveolar type II cells necessary for the production of surfactant. During the alveolar phase secondary septa grow into the airspaces partitioning the more primitive pouches into alveolar ducts and sacs. When fully developed, the structure of the alveoli is analagous to a square room with one wall missing, where they open directly into respiratory bronchioles or alveolar ducts and sacs.

Major Cells of the Lower Respiratory Tract
(Adapted from reference (10))

Cell Type	**Morphology**	**Functions**	**Location**
Ciliated	Columnar, tapering toward the base; glycogen lakes in cytoplasm; ~250 cilia on apical surface	Proximal transport of the mucus stream	Bronchi and bronchioles
Clara	Columnar non-ciliated; copious smooth endoplasmic reticulum; protruding apical cap containing secretory-like dense granules	Secretion of components extracellular lining fluid; metabolism and detoxification of toxins; renewal of bronchiolar epithelium; secretion of surfactant proteins (reviewed in Widdicombe and Pack 1982 (103))	Predominantly in distal conducting airways
Goblet	Columnar, tapering toward the base; contain electron lucent, finely granular mucin	Secretion of mucus	Predominantly trachea and bronchi (proximal airways)

	droplets that discharge apically;		
Basal	Small round to polygonal cells situated along the basal lamina	Possible progenitor of ciliated and goblet cells; regulation of inflammation	Bronchi and bronchioles; more numerous proximally
Neuroendocrine	Triangular cells broadest at base and tapering apically; cytoplasm contains dense core granules; solitary or in clusters (neuroepithelial bodies, NEBs)	Specific function unknown; regarded as an oxygen sensitive chemoreceptor with regulatory functions (reviewed in Van Lommel et al. 1999 (99))	Trachea, bronchi and bronchioles
Oncocytic	Eosinophilic mitochondria-rich cells	Ion secretion	Trachea and ducts of submucosal glands
Squamous	Stratified epithelium as an abnormal reaction replacing normal pseudo-stratified respiratory epithelium	Protective, reparative	Bronchi and bronchioles
Alveolar type I	Large, flat, squamoid cells with few organelles and thin cytoplasm; cover ~93% of alveolar surface area; non-dividing	Air/blood gas exchange	Alveoli
Alveolar type II	Domed cuboidal cells containing lamellar bodies(surfactant storage sites); comprise 60% of pneumocyte population but cover only ~5% of alveolar surface	Proliferate in response to lung injury acting as progenitor for type I cells; synthesize, secrete and recycle surfactant (reviewed in Fehrenbach 2000 (17))	Alveoli

For additional information about lung development and cells of the pulmonary epithelium see:

Histology of the Respiratory System
An Overview of Lung Development
Respiratory Portion (pnuemocytes)

Murine lung development begins at embryonic day 9 (E9) when the lung bud arises as an evagination from the primitive esophagus. Murine lung development has been divided into four chronological stages similar to the stages of human lung development. First is the pseudoglandular stage that lasts from E9.5-E16.6, during which the bronchial and respiratory tree develops, giving rise to a four lobed right lung and a one lobed left lung. During the canalicular stage (E16.6-E17.4) terminal sacs and vascularization develop. The terminal sac stage occurs from E17.4-P5 during which the number of terminal sacs and extent of vascularization increases and the alveolar type I and type II cells differentiate. Lung development is completed during the alveolar stage, P5-P30, when the terminal sacs develop into mature alveolar ducts, sacs and alveoli (101).

The murine pulmonary epithelium is simpler than that of the human. The cell lineages of the airway epithelium are organized in a distinct proximal-distal pattern. The upper airways are lined with ciliated columnar cells and mucus secreting cells, while the lower airways are lined mainly with Clara cells.

Tumor Classification and Staging

The classification of lung tumors is based on the microscopic appearance of hemotoxylin and eosin stained tumor sections. The World Health Organization (WHO) tumor classification system is the most widely accepted schema for the histological typing of lung tumors.

WHO Histologic Classification of Lung Tumors
(Reprinted from Travis et al. 1999 (96))

1. Epithelial Tumors
    - 1.1 Benign
        - 1.1.1 Papillomas
            - 1.1.1.1 Squamous
                - 1.1.1.1.1 Exophytic
                - 1.1.1.1.2 Inverted
            - 1.1.1.2 Glandular papilloma
            - 1.1.1.3 Mixed squamous cell and glandular papilloma
        - 1.1.2 Adenomas
            - 1.1.2.1 Alveolar adenoma
            - 1.1.2.2 Papillary adenoma
            - 1.1.2.3 Adenomas of salivary gland type
                - 1.1.2.3.1 Mucous gland adenoma
                - 1.1.2.3.2 Pleomorphic adenoma
            - 1.1.2.4 Mucinous cystadenoma
    - 1.2 Preinvasive lesions
        - 1.2.1 Squamous dysplasia/carcinoma *in situ*

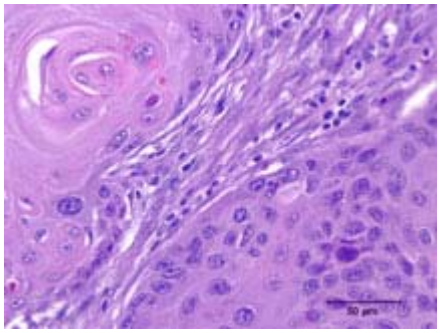
1.2.2 Atypical adenomatous hyperplasia
1.2.3 Diffuse isiopathic pulmonary neuroendocrine cell hyperplasia
1.3 Invasive malignant
1.3.1 Squamous cell carcinoma
Variants:
1.3.1.1 Papillary
1.3.1.2 Clear Cell
1.3.1.3 Small cell
1.3.1.4 Basaloid
1.3.2 Small cell carcinoma
Variant:
1.3.2.1 Combined small cell carcinoma
1.3.3 Adenocarcinoma
1.3.3.1 Acinar
1.3.3.2 Papillary
1.3.3.3 Bronchioloalveolar carcinoma
1.3.3.3.1 Non-mucinous (Clara cell/type II pneumocyte type)
1.3.3.3.2 Mucinous (goblet cell type)
1.3.3.3.3 Mixed mucinous and nonmucinous (Clara cell/type II pneumocyte goblet cell type) or indeterminate
1.3.3.4 Solid adenocarcinoma with mucin formation
1.3.3.5 Mixed
1.3.3.6 Variants:
1.3.3.6.1 Well differentiated fetal adenocarcinoma
1.3.3.6.2 Mucinous (“colloid”)
1.3.3.6.3 Mucinous cystadenocarcinoma
1.3.3.6.4 Signet ring, Clear cell
1.3.6.6.5 Clear cell
1.3.4 Large cell carcinoma
Variants:
1.3.4.1 Large cell neuroendocrine carcinoma
1.3.4.1.1 Combined large cell neuroendocrine carcinoma
1.3.4.2 Basaloid carcinoma
1.3.4.3 Lymphoepithelioma-like carcinoma
1.3.4.4 Clear cell carcinoma
1.3.4.5 Large cell carcinoma with rhabdoid phenotype
1.3.5 Adenosquamous carcinoma
1.3.6 Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements
1.3.6.1 Carcinomas with spindle and/or giant cells
1.3.6.1.1 Pleomorphic carcinoma
1.3.6.1.2 Spindle cell carcinoma
1.3.6.1.3 Giant cell carcinoma
1.3.6.2 Carcinosarcoma
1.3.6.3 Blastoma (pulmonary blastoma)
1.3.6.4 Others
1.3.7 Carcinoid tumors

- 1.3.7.1 Typical carcinoid
- 1.3.7.2 Atypical carcinoid
- 1.3.8 Carcinomas of salivary gland type
- 1.3.8.1 Mucoepidermoid carcinoma
- 1.3.8.2 Adenoid cystic carcinoma
- 1.3.8.3 Others
- 1.3.9 Unclassified carcinoma

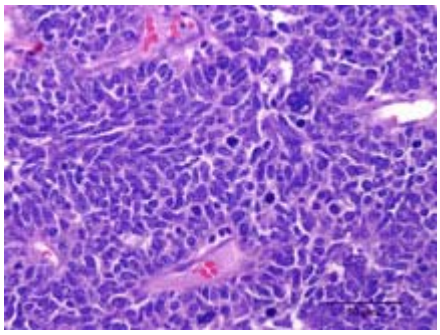
2. Soft tissue tumors
   - 2.1 Localized fibrous tumor
   - 2.2 Epithelioid hemangioendothelomia
   - 2.3 Pleuropulmonary blastoma
   - 2.4 Chondroma
   - 2.5 Calcifying fibrous pseudotumor of the pleura
   - 2.6 Congenital peribronchial myofibroblastic tumor
   - 2.7 Diffuse pulmonary lymphangiomatosis
   - 2.8 Desmoplastic small round cell tumor
   - 2.9 Other
3. Mesothelial tumors
   - 3.1 Benign
     - 3.1.1 Adenomatoid tumor
   - 3.2 Malignant
     - 3.2.1 Epithelioid mesothelioma
     - 3.2.2 Sarcomatoid mesothelioma
       Desmoplastic mesothelioma
     - 3.2.3 Biphasic mesothelioma
   - 3.3 Other
4. Miscellaneous tumors
   - 4.1 Hamartoma
   - 4.2 Sclerosing hemangioma
   - 4.3 Clear cell tumor
   - 4.4 Germ cell neoplasms
     - 4.4.1 Teratoma, mature
     - 4.4.2 Teratoma, immature
     - 4.4.3 Other germ cell tumors
   - 4.5 Thymoma
   - 4.6 Melanoma
   - 4.7 Others
5. Lymphoproliferative diseases
   - 5.1 Lymphoid interstitial pneumonia
   - 5.2 Nodular lymphoid hyperplasia
   - 5.3 Low-grade marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue
   - 5.4 Lymphomatoid granulomatosis
6. Secondary tumors
7. Unclassified tumors
8. Tumor-like lesions

8.1 Tumorlet
8.2 Multiple meningotheloid nodules
8.3 Langerhans' cell histiocytosis
8.4 Inflammatory pseudotumor (inflammatory myofibroblastic tumor)
8.5 Organizing pneumonia
8.6 Myeloid tumor
8.7 Hyalinizing granuloma
8.8 Lymphangioleiomyomatosis
8.9 Multifocal micronodular pneumocyte hyperplasia
8.10 Endometriosis
8.11 Bronchial inflammatory polyp
8.12 Others

The different types of lung carcinoma are characterized by unique histopathologic features that enable their differential diagnosis. Squamous cell carcinoma is a malignant eptihelial tumor exhibiting features of squamous epithelium including keratinzation, intercellular bridge or both (10). Tumor cells are characterized by eosinophilic or clear cytoplasm and shrunken nuclei. The nuclei are hyperchromatic with coarse chromatin and may have prominent nucleoli (23).



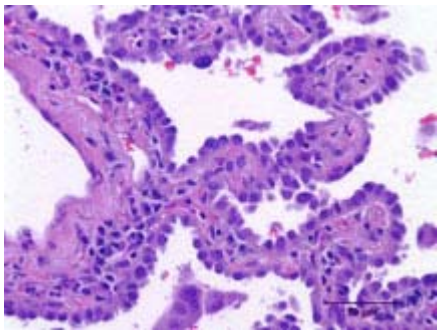
Diagnosis: Squamous carcinoma of the lung.
Species: Human
Boston reference set number: LW031



Small cell carcinoma is a neuroendocrine tumor. Neuroendocrine cells are nonciliated, cylindrical cells in the basal mucosa that contain electron-dense neurosecretory granules (10). Histologically, small cell carcinomas are characterized by small cells (larger than lymphocytes) with a high nuclear to cytoplasmic ratio. Nuclei exhibit finely granular chromatin, inconspicuous nucleoli and nuclear molding. Tumors often exhibit high mitotic rates.

Diagnosis: Small cell carcinoma of the lung.
Species: Human
Boston reference set number: LW036

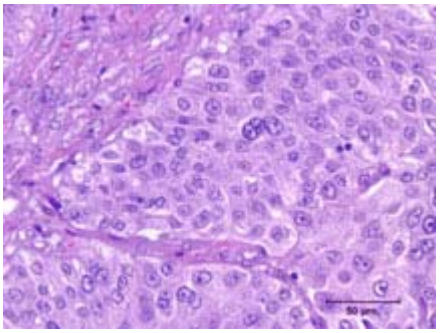
Adenocarcinoma is a glandular epithelial malignancy displaying growth patterns that are either tubular, acinar, papillary or solid with mucin production. They are diverse tumors with varying degrees of differentiation, even within an individual tumor. The tumor cells are large columnar or cuboidal cells with prominent nuclear membranes and nucleoli. Lesions often display fibrous stroma and tumors may be associated with an apical scar (23). The growth patterns of adenocarcinoma result in destruction of the underlying alveolar architecture. Bronchioloalveolar carcinoma (BAC) is a subtype of adenocarcinoma that it quite distinctive in that it is well differentiated, results in minimal architectural destruction, and does not induce a stromal response.



Diagnosis: Bronchioloalveolar carcinoma of the lung.
Species: Human
Boston reference set number: LW035

Large cell carcinoma is a malignant epithelial tumor whose differential diagnosis is based upon exclusion of other tumor types. It is defined by large cells with abundant cytoplasm, without glands, keratin, intercellular bridges or other characteristic features of squamous cell, small cell or adenocarcinoma. Tumors most typically grow as sheets or nests of large polygonal cells containing large vesicular nuclei with prominent nucleoli. However, there is a wide spectrum of histological characteristics among different large cell carcinomas.

The clinical course of disease varies with the different subtypes of lung carcinoma and often reflects the stage of disease at the time of diagnosis. The currently accepted staging system for lung carcinoma is a TNM-based system where T refers to the size of the primary tumor; N, to the involvement of regional lymph nodes; and M to the presence of distant metastasis. It was proposed by Mountain in 1986 and was revised in 1997 to give the current International Staging System for Lung Cancer (64). Stage grouping is performed based on a patients TNM descriptors in order to combine subsets of patients classified according to TNM descriptors into categories that have generally similar treatment options and survival expectations (64).



Diagnosis: Large cell carcinoma of the lung.
Species: Human
Boston reference set number: LW060

TNM Descriptors
(Reprinted from Mountain 1997 (64))

Primary tumor (T)

TX Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

T0 No evidence of primary tumor

Tis Carcinoma *in situ*

T1 Tumor $\leq$ 3 cm in greatest dimension, surrounded by lung or visceral pleura, without Bronchoscopic evidence of invasion more proximal than the lobar bronchus* (*i.e.*, not in the main bronchus)

T2 Tumor with any of the following features of size or extent:

- > 3 cm in greatest dimension
- Involves main bronchus, $\geq$ 2 cm distal to the carina
- Invades the visceral pleura
- Associated with atelectasis or obstructive pneumontitis that extends to the hilar region but does not involve the entire lung.

T3 Tumor of any size that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium, or tumor in the main bronchus < 2 cm distal to the carina, but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung

T4 Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; or tumor with a malignant pleural or pericardial effusion,† or with satellite tumor nodule(s) within the ipsilateral primary-tumor lobe of the lung

Regional lymph nodes (N)

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis to ipsilateral peribronchial and/or ipsilateral hilar lymph nodes, and intra-pulmonary nodes involved by direct extension of the primary tumor

N2 Metastasis to ipsilateral mediastinal and/or subcarinal lymph node(s)

N3 Metastasis to contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

Distant metastasis (M)

MX Presence of distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis present⊥

* The uncommon superficial tumor of any size with its invasive component limited to the bronchial wall, which may extend proximal to the main bronchus, is also classified T1

† Most pleural effusions associated with lung cancer are due to tumor. However, there are a few patients in whom multiple cytopathologic examinations of pleural fluid show no tumor. In these cases, the fluid is nonbloody and is not an exudate. When these elements and the patient's disease judgment dictate that the effusion is not related to the tumor, the effusion should be excluded as a staging element and the patient's disease should be staged T1, T2, or T3. Pericardial effusion is classified according to the same rules.

⊥ Separate metastatic tumor nodule(s) in the ipsilateral nonprimary-tumor lobe(s) of the lung are also classified M1.

Stage Grouping-TNM *Subsets*
(Reprinted from Mountain 1997 (64))

Stage	TNM Subset
0	Carcinoma *in situ*
IA	T1N0M0
IB	T2N0M0
IIA	T1N1M0
IIB	T2N1M0
	T3N0M0
IIIA	T3N1M0
	T1N2M0
	T2N2M0
	T3N2M0
IIIB	T4N0M0
	T4N1M0
	T4N2M0
	T1N3M0
	T2N3M0
	T3N3M0
	T4N3M0
IV	Any T Any N M1

The TNM staging system does not apply well to small cell carcinomas. A two-stage system including limited and extensive disease is favored.

Two-Stage Classification Small Cell Carcinoma
(Reprinted from Colby et al. 1995 (10))

Limited Disease (30 percent of cases)
- Primary tumor confined to hemithorax
- Ipsilateral hilar nodes
- Ipsilateral and contralateral supraclavicular nodes
- Ipsilateral and contralateral mediastinal nodes
- Pleural effusion

Extensive disease (70 percent of cases)
- More advanced than limited disease
- Metastases to contralateral lung
- Distant Metastases

Molecular Alterations (reviewed in 22, 78, 81, 91))

Oncogenes and Signal Transduction

The Ras family of proto-oncogenes encodes a family of small GTPase proteins that transduce proliferation and survival signals from RTKs at the cell membrane. Activating mutations in the K-ras proto-oncogene are found in about 20-50% of NSCLCs, especially adenocarcinomas, and are associated with smoking (76). Point mutations at codon 12 are the most frequent, followed by mutations at codons 13 and 61, and result in a decreased intrinsic GTPase activity and inappropriate constitutive signaling for cell proliferation.

The Myc proto-oncogene family encodes three basic-helix-loop-helix transcription factors, c-Myc, N-myc and L-Myc. The MYC proteins regulate the expression of key cell cycle regulators and genes involved in DNA synthesis and RNA metabolism. Activation of MYC occurs through gene amplification or transcriptional dysregulation, both resulting in over expression of the MYC protein. Results of numerous studies of Myc gene amplification have shown that one Myc family member is amplified in 18-38% of SCLCs and 8-20% of NSCLCs with the lower end of the range representing findings in primary tumors and the upper end in cell lines (75). Furthermore, Myc mRNA expression has been noted in 33-67% of NSCLCs (41). Myc family DNA amplification has been associated with the highly malignant variant class of SCLC (V-SCLC) (50). It is also seen more often in patients that have been previously treated than in untreated patients, and is associated with reduced survival (41).

Autocrine/Paracrine Loops

Many growth factors or neuropeptides and their cognate receptors are expressed by individual cancer cells or the adjacent stroma. This results in several autocrine or paracrine loops that provide a driving force for tumor cell proliferation. Autocrine loops involving co-expression of neuropeptides and their specific G-protein coupled receptor (GPCR) are especially common in SCLC. Activated GPCRs have been shown produce proliferative signals and to elicit a mitogenic response in a variety of cell types (reviewed in (35)). Bombesin/gastrin releasing peptide (GRP), bradykinin, cholecystokinin (CCK),

gastrin, neurotensin and vasopressin are all thought to be involved in driving SCLC growth (78).

One well-characterized autocrine system involves gastrin-releasing peptide or other bombesin-like peptides (GRP/BN) and their receptors. Expression of GRP was demonstrated in 20-60% of SCLC by immunohistochemical analysis. Neutralizing antibodies against GRP/BN and bombesin antagonists inhibit both in vitro and in vivo growth of SCLC cell lines, and monoclonal antibodies show anti-tumor activity against SCLC in clinical trials (81). Thus GRP/BN autocrine signaling appears to play an important role in stimulating growth of SCLCs. Interestingly, the aberrant expression of these genes does not seem to be linked to gene amplification or rearrangement. GRP/BN is known to be involved in embryonic lung development suggesting that perhaps the cells of these tumors have de-differentiated to a more primitive state or have reactivated developmentally important signaling pathways. In addition the high percentage (57%) of SCLCs expressing both gastrin and its receptor CCK-B (74) further support a prominent role of neuropeptide autocrine signaling as a driving force for SCLC proliferation.

Peptide growth factor autocrine loops are more commonly found in NSCLC than SCLC. These growth factors bind to and activate receptor tyrosine kinases (RTKs) that then initiate intracellular signaling cascades. Expression of the Neuregulin receptor ERBB2 (also known as HER2/*neu*) has been noted in ~30% of NSCLCs and has been associated drug resistance and metastatic potential. The ERBB1 receptor (also known as the Epidermal Growth Factor Receptor) is also commonly over expressed in NSCLC along with its ligands TGF-a, amphiregulin and EGF (78),(81). Several additional growth factor/RTK autocrine loops may also play a role in SCLC lung cancer proliferation including KIT and its ligand stem cell factor (SCF) as well as insulin like growth factors (IGFs) and their receptor IGF-R that are expressed in both SCLC and NSCLC.

Anti-apoptotic Genes

Bcl-2 was first identified as a proto-oncogene located at a translocation breakpoint in many B cell lymphomas. Bcl-2 is an anti-apoptotic protein that functions at the mitochondrial membrane. It is thought to promote cell survival by inhibiting linker proteins necessary for the activation of caspases. More than 90% of SCLCs express the bcl-2 protein. Most Bcl-2 positive tumors express the protein in a high percentage of the tumor cells (40, 109). A smaller subset of NSCLCs is bcl-2 positive. The bcl-2 protein is expressed in ~25% of squamous cell carcinomas and ~12% of adenocarcinomas (68). Interestingly, bcl-2 expression is thought to correlate with good prognosis in NSCLCs, but does not seem to correlate with prognosis in SCLC.

Tumor Suppressor Genes and Cell Cycle Regulation

Mutations or deletions of p53 are very common in both NSCLC (50%) and SCLC (80%). P53 normally acts to induce cell cycle arrest or apoptosis in response to cellular stresses such as DNA damage. P53 functions as a sequence specific transcription factor activating genes responsible for G1 arrest such as $p21^{Waf1/Cip1}$, to allow cells to repair damaged DNA before replication. Alternatively p53 can activate the transcription of genes involved in apoptosis such as BAX and PERP others. Mutations in the p53 gene are found in ~70% of SCLC and ~45% of NSCLC (9, 31, 88).

Alterations in the Rb pathway are also important in both SCLC and NSCLC. The RB protein acts as a growth suppressor by inactivating proteins that promote transcription of genes required for DNA replication, thus blocking the G1/S transition. RB mutations are found in up to 90% of SCLCs and 15-30% of NSCLCs most of which result in a truncated RB protein(6, 14, 36, 73). Furthermore, Cyclin D1 is over expressed in up to 47% of NSCLCs (4, 60). Cyclin D1 acts to inhibit RB function by inducing it phosphorylation by Cdk4. Mutations in p16INK4A, an inhibitor of Cdk4 kinase activity, are also common in NSCLC (~60%)(67).

A second protein p14ARF is encoded by the p16INK4A locus. p14ARF is transcribed from an alternate reading frame that largely overlaps that of p16INK4A, but results in a totally unrelated protein. p14ARF prevents p53 degradation by MDM2, resulting in p53 activation. p14ARF mutations are found in 19-37% of NCLCs (25, 66). The p14ARF protein is frequently lost in SCLC (65%), although the mRNA transcript is still present suggesting a post-transcriptional mechanism of inactivation. Interestingly, loss of p14ARF often occurs in the presence of p53 mutations in both NSCLC and SCLC, suggesting an alternative tumor suppressor function for p14ARF, distinct from that of p16INK4A(25).

Common regions of chromosomal loss and LOH suggest the existence of other tumor suppressor genes involved in lung tumorigenesis. Deletions of 3p are observed in 50% of NSCLCs and 90% of SCLCs . Such deletions often include FHIT, a candidate TSG, and abnormal FHIT mRNAs have been found in 40-80% of lung cancer (85). However many of these tumors also express the wild type FHIT transcript as well, raising the question of whether FHIT acts as a classical tumor suppressor. Additional sites of chromosomal loss for which the candidate TSGs remain unknown include 4p, 4q, 5p, 5q, 10p, 10q, 13q34 for SCLC; 1p, 6p, 13q11, 18q, 19p and Xq22.1 for NSCLC; and 8p, 9q, Xp for both SCLC and NSCLC (28).

Tumor Vasculature

Small tumors (1-3mm) can obtain nutrients and oxygen by passive diffusion from their surrounding tissues. However, neovascularization is needed to support tumor growth, progression and metastasis. For tumors to induce angiogenesis, tumor cells and stromal cells must secrete factors that induce endothelial migration and proliferation. Vascular endothelial growth factor (VEGF) stimulates neovascularization in a paracrine fashion. It is expressed by >50% of NSCLCs, and is associated with an increase in intratumoral microvascular density (IMD) and poor prognosis (59), (100). Platelet derived endothelial growth factor (PD-ECGF) was initially identified as a novel angiogenic factor in platelets (98). PD-ECGF is expressed by ~32% of squamous cell carcinomas, 42% of adenocarcinomas and 33% of adenosquamous carcinomas (26). IL-8 is a member of the CXC chemokine family and has been reported to be a potent angiogenic factor (46). IL-8 is expressed in approximately 45% of NSCLCs and is associated with increased IMD (59). However, neither IL-8 nor PD-ECGF is expressed at significant levels in SCLC (110), (108). Finally, 49-70% of pulmonary adenocarcinomas express bFGF and expression correlates with poor prognosis (89, 90).

Novel Therapeutics

The discussion below provides information on molecular alterations that occur frequently in lung cancer (see above for in depth discussion) and to give some examples of therapeutic agents that target them. There may be additional targeted therapeutics that have not been described below. For a more complete listing of drugs in development and ongoing clinical trials please use the following links:
Cancer.gov Clinical Trials

Treatment approaches for lung cancer depend upon the stage of disease at the time of diagnosis. For patients diagnosed with early stage lung cancer (Stage I and II) surgery is the treatment of choice. More often patients are diagnosed with advanced stage disease in which the cancer has spread to the mediastinal lymph nodes (Stage III) or to distant organs (Stage IV). In such cases patients are treated with a combination of radiotherapy and chemotherapy (Stage III) or with chemotherapy alone (Stage IV). However, approximately 90% of patients diagnosed with advanced disease succumb to a recurrence of chemotherapy resistant, metastatic disease (80). Over the past twenty years efforts to lengthen the median survival time for patients diagnosed with lung cancer have focused largely on the use of new combinations of cytotoxic agents, and have resulted in only minimal improvements in survival time. Recent advances in our understanding of the molecular alterations underlying the biology of lung cancer have led to the development of rationally targeted therapies as a new approach to the treatment of lung cancer.

Peptide growth factor autocrine loops are commonly found in NSCLCs. The cells of these tumors often inappropriately express high levels of receptor tyrosine kinases (RTKs) that drive cell proliferation upon binding of their ligands. The epidermal growth factor receptor (EFGR), also known as the ERBB1 receptor, is expressed by approximately 30% of NSCLCs, and has thus been the target of many rationally designed therapeutics. Iressa (ZD1839) is an orally available, quinazolone compound that selectively inhibits ligand induced EFGR autophosphorylation by competitive inhibition of ATP binding. Phase I clinical trials showed Iressa to be well tolerated at doses that inhibited EGFR function in biomarker studies (51). Iressa is currently in Phase II and III studies for the treatment of lung, head and neck, colon and breast cancer. C225 represents an alternative approach to EGFR inhibition. It is a monoclonal antibody directed against the extracellular domain of EGFR. It was shown to be well tolerated and to have anti-cancer activity in Phase I trials (82), and is currently in Phase II trials for the treatment of NSCLC, head and neck and colon cancer . OSI-774 is an orally available small molecule inhibitor of EFGR that has also shown promising results in Phase I and II trials, with Phase III trials being planned (82).

Neovascularization is required for tumors to grow beyond 3mm in diameter, and for metastasis. Several inhibitors of angiogenesis are currently being studied for effectiveness in the treatment of lung cancer. A recombinant humanized monoclonal antibody to VEGF (Bevacizumab) has been developed by Genentech, Inc. Efficacy has been evaluated in NSCLC patients in Phase II trials accompanying chemotherapeutic treatment and showed an increased response rate and median survival (82). Phase III studies are currently under development. SU5416, produced by Sugen, is a synthetic

antagonist of the VEGF receptor, Flk-1. Phase I studies are currently underway to evaluate toxicity when SU5416 is given with paclitaxel and carboplatin. Additional small molecule inhibitors of angiogenesis are currently in early development for lung cancer treatment including; SU6668, an orally administered small molecular inhibitor of RTKs including Flk-1, PDGF receptor and FGF-1, and ZD4190 a VEGF RTK inhibitor produced by AstraZeneca (82). Furthermore, several matrix metalloproteinase (MMP) inhibitors are being studied for efficacy in the treatment of both SCLC and NSCLC. Marimastat (BB2516), a synthetic MMP inhibitor produced by British Biotech, is currently in Phase III trials for the treatment of SCLC and Stage III NSCLC. BMS-275291 is a synthetic MMP inhibitor produced by Bristol-Myers Squibb. It is in Phase II/III trials for advanced NSCLC. Neovastat is a naturally occurring MMP inhibitor extracted from shark cartilage, is undergoing Phase II/III clinical trials for the treatment of advanced NSCLC.

Proliferation of SCLCs is often driven by autocrine loops involving neuropeptides and their cognate G protein coupled receptor (GPCR). Binding of neuropeptide to its receptor results in activation of the associated G protein and production of second messengers which go on to activate several downstream signaling pathways. The production of two such second messengers, inositol triphosphate and diacylglycerol (DAG) result in the activation of protein kinase C (PKC). PKC then activates several other signal cascades involved in the regulation of cellular proliferation. PKC is also thought to function downstream of RTKs, and thus may also be involved in autocrine loops stimulating the growth of NSCLC. Furthermore, the cancer promoting agent phorbol ester is present as an air pollutant and may be important in the induction of lung cancer, especially that of the squamous cell type. Phorbol ester is a DAG analogue, and induces tumor formation through activation of PKC (8). Thus PKC may be an important therapeutic target in the treatment of lung cancer. ISIS 3521, an antisense inhibitor of PKC-Alpha has shown promising results in Phase I/II trials for the treatment of NSCLC (16); A Phase III clinical trial is ongoing.

Inactivating mutations of the p53 tumor suppressor gene are very common in both SCLC (80%) and NSCLC (50%). The p53 protein normally functions to induce cell cycle arrest or apoptosis in response to DNA damage, oncogene expression and other cellular stresses. Loss of p53 function also correlates with increased resistance to radiation and chemotherapy. Adenoviral p53 gene therapy was shown to be well tolerated and to have significant clinical activity for the treatment of advanced NSCLC when injected into a single tumor (77, 102) and is also being tested when delivered by bronchoalveolar lavage. Furthermore, Phase II trials are underway to assess the efficacy of immunotherapy with mutant p53 peptide-pulsated autologous dendritic cells in treating NSCLC patients with appropriate p53 mutations. Additional trials on tumor specific p53 peptide vaccines are also underway in patients with advanced cancers.

Activating mutations of the K-ras oncogene occur in approximately 30% of pulmonary adenocarcinomas. Point mutations at codons 12, 13 and 61 of the ras genes result in proteins with decreased intrinsic GTPase activity leading increased signaling of downstream effector proteins regulating cell proliferation, survival and differentiation. Phase I studies are being conducted to test ras peptide cancer vaccines in which NSCLC patients receive mutant peptide vaccines specific to the mutation in their tumors. The ras proteins must be localized to the cell membrane in order to function in the cell. Such

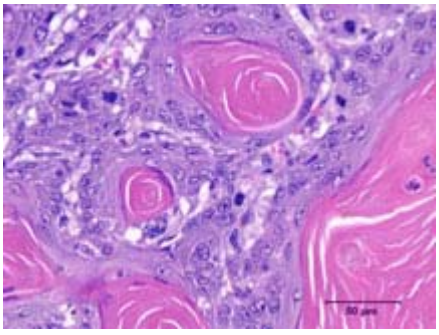
localization occurs through post translation prenylation of the protein, including farnesylation and geranylgeranylation (45). Farnesyl protein transferase (FT) is the enzyme that catalyzes the transfer of a farnesyl moiety to the ras protein, and thus presents an attractive target for inhibiting ras function. The FT inhibitors R115777 is currently in clinical trials for the treatment of advanced NSCLC.

Murine Lung Cancer Models

Several approaches have been taken for creating murine lung cancer models. Specific inbred strains of mice are susceptible to the development of spontaneous lung tumors. The most sensitive strains include A/J and SWR while others range from intermediated sensitivity (BALB/c and O20), somewhat resistant (CBA and C3H) to nearly fully resistant (DBA and C57BL/6). The susceptible strains are also sensitive to chemically-induced lung tumors, and this sensitivity has been employed as a carcinogenicity bioassay (84). A polymorphism in the second intron of K-ras, that may affect gene expression levels, is one major modifier of sensitivity to lung tumorigenesis (111).

These strain differences in tumor susceptibility have been exploited for the mapping of additional loci that confer sensitivity to lung cancer. Analysis of progeny from crosses between recombinant inbred (RI) strains derived from the sensitive A/J strain and the resistant C57BL/6J strain, suggested the existence of three pulmonary adenoma susceptibility (Pas) loci (55). Pas-1 was later identified by analysis of F2 progeny from a cross between strain A/J and the C3H/He resistant strain and was mapped to the distal region of chromosome 6 (24). Linkage analysis has demonstrated K-ras to be tightly linked to the Pas-1 locus, suggesting K-ras as a candidate for Pas-1(47). Additional Pas loci have been mapped to chromosomes 9, 17 and 19 (13, 18). Furthermore, several Numerous susceptibility to lung cancer (Sluc) loci have been identified by using a multilocus mapping method to analyze F2 mice generated from recombinant congenic strains (RCS). The Sluc loci are involved in complex genetic interactions that control susceptibility to the development of lung cancer (19, 20).

A wide variety of chemical carcinogens can induce pulmonary adenoma and adenocarcinoma formation in mice although they vary in their potencies (For a review on spontaneous and chemically induced mouse lung tumors see Shimkin and Stoner 1975; Malkinson 1989; Stoner 1998 (53, 84, 86)). Some well characterized tumorigenic agents include urethane, metals, aflatoxin, tobacco smoke and tobacco smoke constituents including polyaromatic hydrocarbons and nitrosamines. Of note, the only murine models of squamous cell carcinoma existing to date have resulted from direct application of a carcinogen, most commonly MC, by intratracheal instillation (65) or by rigorous topical administration of NMBCU or NTCU twice a week for 35-40 weeks (71). The study of chemically induced lung tumors has

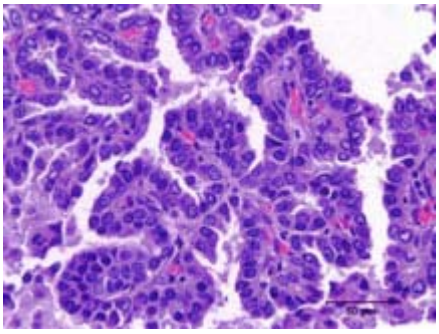


Diagnosis: Keratinizing squamous carcinoma of the lung.
Species: Mouse
Boston reference set number: LW071A

provided insights into the histogenesis of murine lung tumors suggesting that murine pulmonary adenocarcinomas are derived from cells of the alveolar epithelium of the type II cell lineage, or from the bronchiolar epithelium of the Clara cell lineage (34, 70, 72, 92).

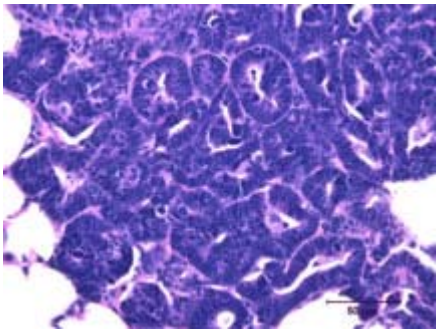
Murine lung tumors are examined both histologically and immunohistochemically in order to gain insights into the histogenesis of the tumors. Anti-SP-C staining is commonly used to identify cells of the alveolar type II cell lineage, while anti-CC10 staining is used to identify cells of the Clara cell lineage. Anti-SPA staining has also been widely used, but is slightly less informative as both Clara cells and type II cells express SP-A. These findings have provided a basis for further investigation into the cell of origin of lung tumors. Furthermore, numerous molecular alterations have been identified in spontaneous and carcinogen induced murine lung tumors including activating mutations of the K-ras oncogene, over expression of c-myc and decreased expression of the Rb tumor suppressor gene (12, 29, 69). Many of these findings correlate with known genetic changes in human lung tumors, suggesting an important role in the formation of pulmonary carcinomas and providing a basis for the development of transgenic models.

Several transgenic lung tumor models have been created in order to direct oncogene expression to a specific subset of lung epithelial cells and to examine the role of specific oncogenes in lung tumorigenesis. Various combinations of oncogenes and cell type specific expression have resulted in murine lung tumors resembling human adenocarcinoma. Expression of SV40 T Antigen from the Clara cell specific CCSP (aka uteroglobulin, UG, or CC10)) promoter or the alveolar type II cell specific SP-C promoter, develop multifocal early onset bronchioloalveolar hyperplasias resulting in death by 4 months of age (11, 79, 104). Mice constitutively over expressing c-myc from the SP-C promoter develop pulmonary lesions ranging from bronchiolo-alveolar adenomas to adenocarcinomas and the age of onset is accelerated in homozygous versus hemizygous mice. However, the penetrance of the phenotype is incomplete, suggesting that the acquisition of additional mutations is necessary for tumorigenesis (15). In addition, the authors demonstrated that mice expressing a secretable form of EGF from the SP-C promoter develop alveolar hyperplasias also with incomplete penetrance. However, when crossed to the SP-C/c-myc animals, 100% of the double transgenic SP-C/c-myc/IgEGF animals develop bronchiolo-alveolar adenocarcinomas, suggesting cooperativity between the oncogenes, and an important role for EGF in promoting lung tumor growth. In order to examine the role of the retinoic acid receptor RARB2 in inhibiting lung tumorigenesis Berard and colleagues (114) created a strain expressing an antisense RARB2 transgene under control of the MMTV promoter. A subset of the mice develop pulmonary adenomas and adenocarcinomas of the lung originating from type II cells or Clara cells as determined by immunohistochemistry.



Diagnosis: Papillary adenocarcinoma of the lung.
Species: Mouse
Boston reference set number: LW003

Additional transgenic strains have elucidated genes whose mutation play a role in lung tumor formation, but are unable to induce tumorigenesis on their own. Mice heterozygous for deletion of the transforming growth factor β1 (TGF-β1) gene show an enhanced rate and increased multiplicity of lung tumorigenesis after treatment with carcinogen (44, 61). Furthermore, transgenic mice over expressing a dominant-negative form of transforming growth factor β receptor type II (TGF-βRII) show an early increase in the incidence of lung tumors after treatment with carcinogen (3).



Diagnosis: Adenocarcinoma with neuroendocrine differentiation.
Species: Mouse
Boston reference set number: LW004

Fewer if any transgenic models exist that resemble human SCLC. Oncogenic H-ras driven from the neuroendocrine specific, calcitonin gene related protein (CGRP) promoter results in the development of both pulmonary neuroendocrine hyperplasia and non-NE tumors described as adenocarcinoma, with the later predominating (87). In an attempt to more closely mimic SCLC, Linnoila and colleagues expressed hASH1, a transcription factor involved in regulating NE differentiation, from the CC10 promoter(49). They found that in the context of coexpression of SV40 T Antigen from the CC10 promoter, CC10-hASH1 expression resulted in NE differentiation of airway epithelial cells, as well as the development of aggressive pulmonary NE carcinomas. Interestingly, CC10-hASH1 alone caused epithelial cell hyperplasia and metaplasia at the bronchioloalveolar junction, but did not result in NE differentiation of these cells. However, although these models develop neuroendocrine tumors, they may more closely resemble human NSCLC with neuroendocrine features than human SCLC.

In humans, lung cancer arises due to the accumulation of mutations in individual cells of the adult lung. The use of conditional alleles of oncogenes and tumor suppressor genes has facilitated the development of murine lung cancer models that more closely mimic the human situation, in which mutations occur in a subset of cells after completion of lung development. Cre/Lox technology has been used to develop conditional alleles of both oncogenes and tumor suppressor genes. Two strains of mice carrying conditional alleles of oncogenic K-ras G12D or K-ras G12V containing a floxed transcriptional stop element have been created. These mice develop pulmonary adenocarcinomas and epithelial hyperplasia of the bronchioles upon infection of the lungs with AdenoCre virus, a recombinant adenovirus expressing the Cre recombinase (38, 62). Lung tumor multiplicity can be regulated in these mice by altering the dose of virus administered. The use of AdenoCre virus to initiate tumorigenesis has facilitated the analysis of tumor progression, as the precise timing of tumor initiation is known. In addition, the use of AdenoCre results in sporadic activation of the K-ras oncogene such that an individual tumor cell is surrounded by normal wild type cells, more closely mimicking the development of human tumors. Another murine lung cancer model based on sporadic K-ras activation was developed using a variation of `hit-and-run' gene targeting (42). These mice carry a latent activatable allele of oncogenic K-ras G12D that is only expressed after

a spontaneous somatic recombination event, resulting in the development of numerous pulmonary adenocarcinomas. The mice also develop intestinal aberrant crypt foci and skin papillomas perhaps reflecting the sensitivity of these tissues to the effects of K-ras mutations.

Several strains of mice have been created that carry floxed alleles of tumor suppressor genes some of which include conditional APC (83), NF1 (113), NF2 (27), Brca1 (5), Brca2 (43), p53 and RB (57). The use of AdenoCre virus or lung specific expression of Cre to inactivate one or a combination of relevant tumor supressor genes may be useful for the creation of additional conditional lung cancer models.

The tetracycline-based bitransgenic regulatable expression system has also been used for the creation of conditional murine lung cancer models. In this system, the reverse tet transactivator (rtTA), under the control of a tissue specific promoter, activates the expression of an oncogenic transgene under the control of the tet-operator. This system enables the investigator to both turn on and turn off oncogene expression at will, by administering or removing doxicycline (a tetracycline-related antibiotic). Both Sp-c-rtTa and Ccsp-rtTA transgenic mice have been created in order to direct expression of the tet-responsive gene to specific cells of the pulmonary epithelium. When Ccsp-rtTA mice are crossed to $(tetO)_7$-CMV-FGF-7 transgenic mice, postnatal administration of doxicycline results in the development of epithelial cell hyperplasia, adenomatous hyperplasia and pulmonary infiltration with mononuclear cells (94). Fisher and colleagues (21) created a $(tetO)_7$-K-ras4b G12D mouse that they crossed to a Ccsp-rtTA trangenic strain. This Ccsp-rtTA strain surprisingly expressed rtTA primarily in alveolar type II cells, presumably due to transgene effects. The Ccsp-rtTA/$(tetO)_7$-K-ras4b G12D bitransgenic mice develop multiple pulmonary adenocarcinomas only after administration of doxicycline. When bred into a background deficient for either the p53 orf Ink4A/Arf tumor suppressor genes, the tumors arise more rapidly and appear more malignant. After withdrawal of doxicycline, the tumors rapidly regress, even in the absence of p53 or Ink4A, demonstrating that activated K-ras is necessary for both tumor initiation and maintenance(21).

MODEL DESIGN	PHENOTYPE
Spontaneous:Strain A/J Strain SWR	Adenomas
Carcinogen Induced: NMBCU or NTCU Topical application	Squamous cell carcinomas
Carcinogen Induced: MC intratracheal instillation	Squamous cell carcinomas

Carcinogen Induced: Numerous carcinogens including: Urethane, PAHs, nitrosamines, aflatoxin B1, NNK, cigarette smoke and others	Adenomas/Adenocarcinomas
Transgenic: CCSP-TAg	Multifocal early onset bronchioloalveolar hyperplasias
Transgenic: SP-C-TAg	Adenocarcinomas including papillary, solid and bronchioloalveolar subtypes
Transgenic: SP-C-myc	Pulmonary tumors ranging from bronchioloalveolar adenomas to adenocarcinomas. Phenotype shows incomplete penetrance.
Transgenic: SP-C-IgEGF	Alveolar hyperplasias. Incomplete penetrance
Bi-Transgenic SP-C-myc;SP-C-IGEF	Bronchioloalveolar adenocarcinomas
Transgenic: MMTV-antisense RARB2	Pulmonary adenomas and adenocarcinomas. Incomplete penetrance
Transgenic: CGRP-H-ras	Pulmonary neuroendocrine (NE) hyperplasia and non – NE adenocarcinomas
Transgenic: CC10-hASH1	Epithelial cell hyperplasia and metaplasia at bronchioloalveolar junction
Bi-Transgenic: CC10-hASH1;CC10-TAg	NE differentiation of airway epithelial cells and pulmonary NE carcinoma.
Conditional Transgenic: Lox-GFP-lox/B-actin-K-ras G12V/IRES/PLAP with intratracheal administration of AdenoCre	AAH, adenomas and adenocarcinomas
Conditional Knock In: Lox-Stop-Lox/K-ras G12D with intranasal administration of $CaPO_4$ AdenoCre coprecipitates	Epithelial hyperplasia of the bronchioles, AAH, adenomas and adenocarcinomas of both solid and papillary subtypes
Dox Regulatable: Transgenic tetO-CMV-FGF7; CCSP-rtTA	Epithelial cell hyperplasia and adenomatous hyperplasia
Dox Regulatable: Transgenic tetO-K-ras 4b G12D; CCSP-rtTA alone and in p53 of Ink4 deficient background	Bronchogenic adenocarcinomas. Reversible with removal of Dox.
Spontaneously activatable: Knock in K-ras $G12D^{LA}$	AAH, adenomas and adenocarcinomas of both solid and papillary subtypes
Spontaneously activatable: Knock in K-ras $G12D^{LA}$; p53 Knock out	AAH; adenomas and adenocarcinomas with shorter latency

Classification of Murine Lung Tumors

Rapid advances in murine lung cancer modeling provide continuous challenges for pathologists. The issue of murine lung tumor classification was addressed at the MMHCC workshop on mouse models of lung cancer held in Boston on June 20-22,2001. Here, a panel of human, veterinary and experimental pathologists devised a new system for the classification of murine lung tumors specifically designed to accommodate appearances of novel nosological units, and to provide guidelines for the comparison of human and mouse lesions. A draft version of this classification system is provided below.

Classification of Proliferative Lesions of the Lung in Mice

(Alexander Yu. Nikitin, Miriam Anver, Roderick Bronson, Robert D. Cardiff, Armando E. Fraire, Edward Gabrielson, William T. Gunning, and Sabine Rehm)

1. Epithelial
1.1 Hyperplasia
1.1.1 Epithelial
1.1.2 Neuroendocrine
1.2 Tumors
1.2.1 Benign
1.2.1.1 Papilloma
1.2.1.2 Adenoma
1.2.1.2.1 Papillary
1.2.1.2.2 Solid
1.2.1.2.3 Adenoma with mixed subtypes
1.2.2 Preinvasive Lesions
1.2.2.1 Squamous dysplasia
1.2.2.2 Atypical adenomatous hyperplasia
1.2.3 Malignant
1.2.3.1 Squamous cell carcinoma
1.2.3.2 Adenocarcinoma
1.2.3.2.1 Papillary
1.2.3.2.2 Acinar
1.2.3.2.3 Solid
1.2.3.2.4 Mixed Subtypes
1.2.3.2.5 NOS
1.2.3.3 Adenosquamous carcinoma
1.2.3.4 Neuroendocrine carcinoma
1.2.3.5 Carcinoma, other
2. Soft Tissue
3. Mesothelial
4. Miscellaneous

5. Lymphoproliferative
6. Secondary
7. Unclassified
8. Tumor-Like

The lung tumors in existing murine models are typically less aggressive than human tumors, with weaker stromal reaction and fewer metastasis. Thus the differential diagnosis between benign, pre-malignant and malignant tumors is more difficult to make in mice, and a consensus for such diagnosis has not yet been reached. The differential diagnosis of adenocarcinoma is based on the presence of large pleomorphic cells with vesicular nuclei, prominent nucleoli, undifferentiated cytoplasm and frequent mitosis.

The cell of origin of murine pulmonary adenocarcinoma is still debatable, but may prove to be a useful criterion for tumor classification. It remains unclear whether tumors can arise from alveolar type II cells, Clara cells and multipotent stem cells or only from one or a subset of these cell types (1, 34, 38, 52, 58, 70, 72, 92, 93, 104). Immunohistochemical staining is often employed as a means to determine the cell of origin of individual tumors (see protocols). Staining with anti-SP-C antibodies is performed to identify cells of the alveolar type II cell lineage, whereas anti-CC10 antibodies are used to determine Clara cell lineage (see protocols). Historically, SP-A has been used as a marker for type II cells, but it is also expressed by Clara cells, although at lower levels, and thus is not as specific a marker as SP-C. Of note, there is evidence to suggest that tumor cells may have the potential to transdifferentiate or may downregulate expression of CC10 and upregulate expression of SP-A as they progress (39, 48, 105) . Therefore, the reactivity of the tumor may not always accurately reflect the cell of origin of the tumor, however it is useful in assessing the differentiation state of the tumor itself. Enzyme histochemistry is also employed as a means to determine the cell of origin. Clara cells exhibit high levels of both glyceraldehyde-3-phosphate dehydrogenase (G3PD) and succinate dehydrogenase activity whereas alveolar type II cells show only slight activity. Histochemical staining has demonstrated that the enzyme activity of solid adenomas is similar to that of alveolar type II cells, whereas the enzyme activity of papillary adenomas is more like that of Clara cells (34, 92).

Pre-clinical Therapeutics

Pre-clinical testing of lung cancer therapeutics has been largely carried out using xenograft models in which human lung cancer cell lines have been subcutaneously injected into immunodeficient mouse strains. However, xenograft models may not accurately mimic the behavior of lung tumors arising in the cellular microenvironment of the normal lung. Accordingly, xenograft models have a poor record of accurately predicting the clinical efficacy of anticancer agents. Carcinogen induced and genetically modified murine lung cancer models that have been shown to accurately mimic the human disease may provide more predictive models in which to perform pre-clinical testing.

As discussed above, strain A mice are highly susceptible to the development of pulmonary adenocarcinoma after treatment with a variety of chemical carcinogens. Studies testing the efficacy of chemo-intervention with cis-platinum alone or in

combination with indomethacin, metoclopramide or nifedipine demonstrated the usefulness of this model system for evaluating therapeutics (2). The strain A model has also been used to assess the ability of potential chemopreventive agents to protect against the development of carcinogen induced lung tumors (33, 107). Furthermore, studies have been conducted to test the efficacy of both chemotherapeutics and chemopreventives for treating or preventing carcinogen induced lung tumors in F1 mice resulting from the cross of strain A mice to p53 null or transgenic p53 mice (112). This study demonstrates the usefulness of transgenic models for pre-clinical testing. Further examination of existing models, both genetically modified and carcinogen induced, with conventional drugs will provide additional support for their predictive value.

The limited success of lung cancer treatment with classic chemotherapeutic agents has led researchers to focus on the development of targeted therapeutics aimed at the molecular mechanisms underlying lung tumorigenesis. Genetically modified mouse models may prove to be extremely well suited for pre-clinical testing of compounds aimed at inhibiting the particular genetic alterations driving tumor formation in a given model. Genetically modified murine cancer models have been used to examine the efficacy of some targeted therapeutics. For example, farnesyl transferase inhibitors that act to inhibit Ras signaling have been tested in several models whose genetic modifications result in upregulation of ras signaling. These studies demonstrated that FTIs are effective for treating some, but not all tumor types (reviewed in (115)). These findings illustrate the importance of testing novel lung cancer therapeutics in well defined lung cancer models.

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